

Figure 1: Size exclusion chromatography (SEC) of heat-stressed MAb A.

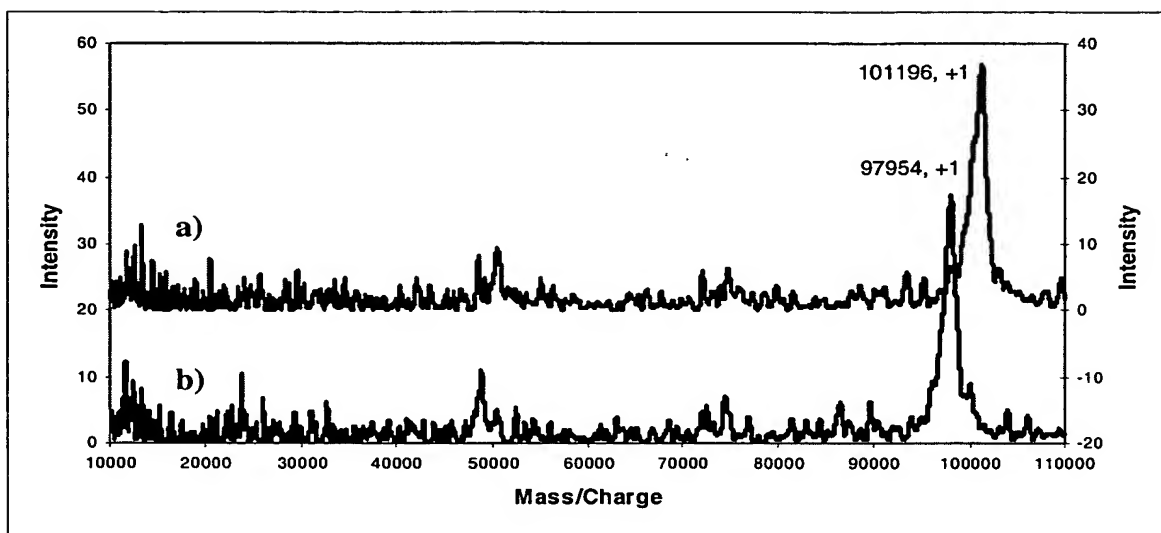


Figure 2: MALDI-TOF-MS mass spectra of a) non-deglycosylated and b) deglycosylated SEC fraction 'Peak 1' of the heat-stressed MAb A.

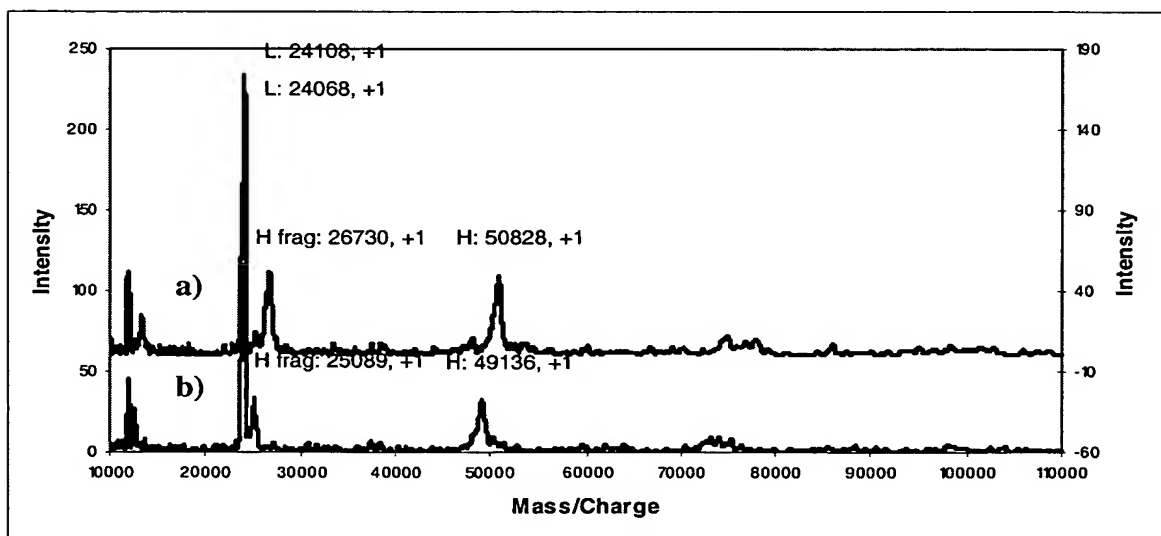


Figure 3: MALDI-TOF-MS mass spectra of a) non-deglycosylated and reduced and b) deglycosylated and reduced SEC fraction 'Peak 1' of the heat-stressed MAb A.

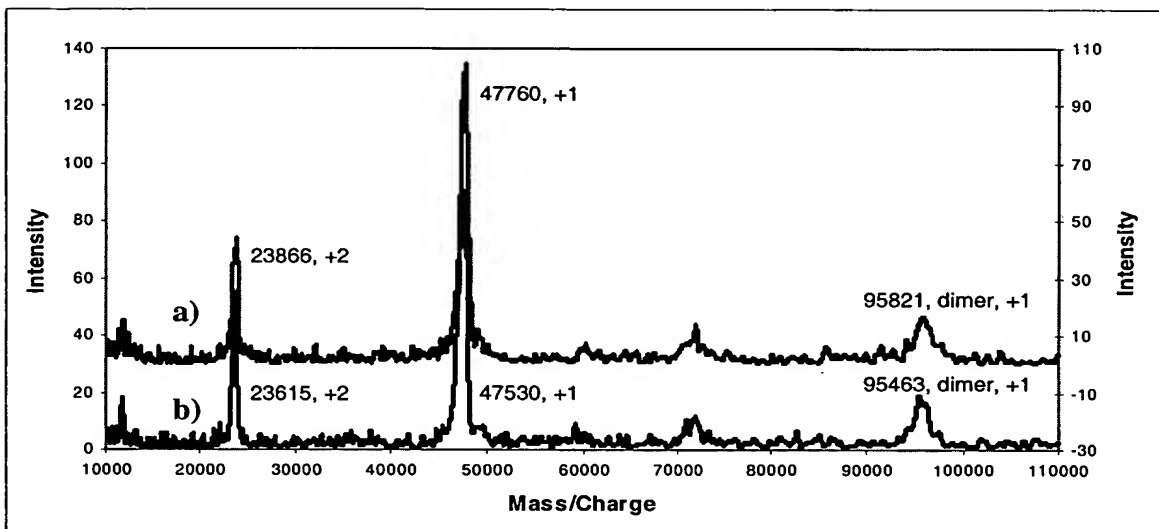


Figure 4: MALDI-TOF-MS mass spectra of a) non-deglycosylated and b) deglycosylated SEC fraction 'Peak 2' of the heat-stressed

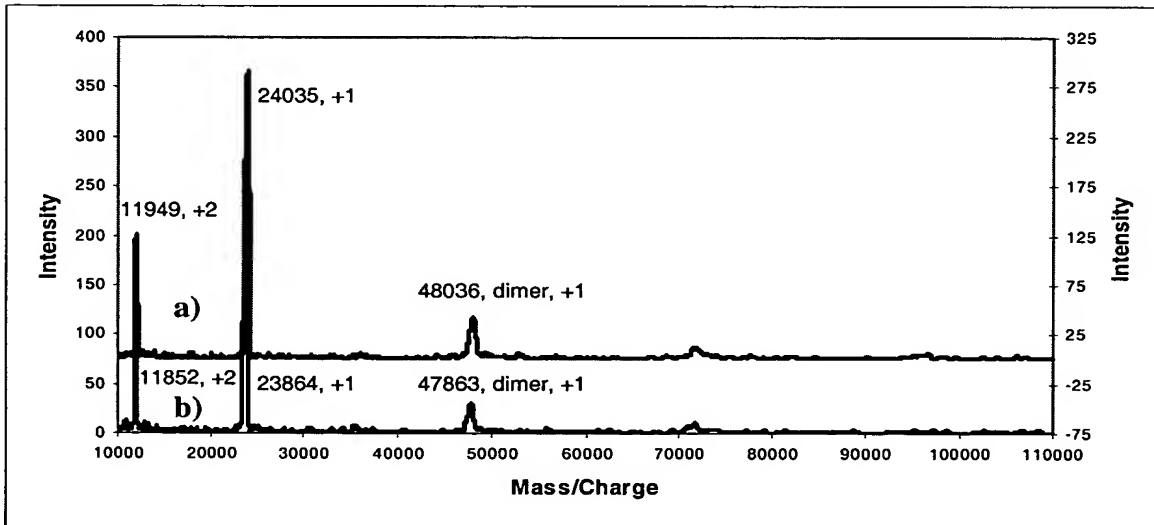


Figure 5: MALDI-TOF-MS mass spectra of a) non-deglycosylated and reduced and b) deglycosylated and reduced SEC fraction 'Peak 2' of the heat-stressed MAb A.

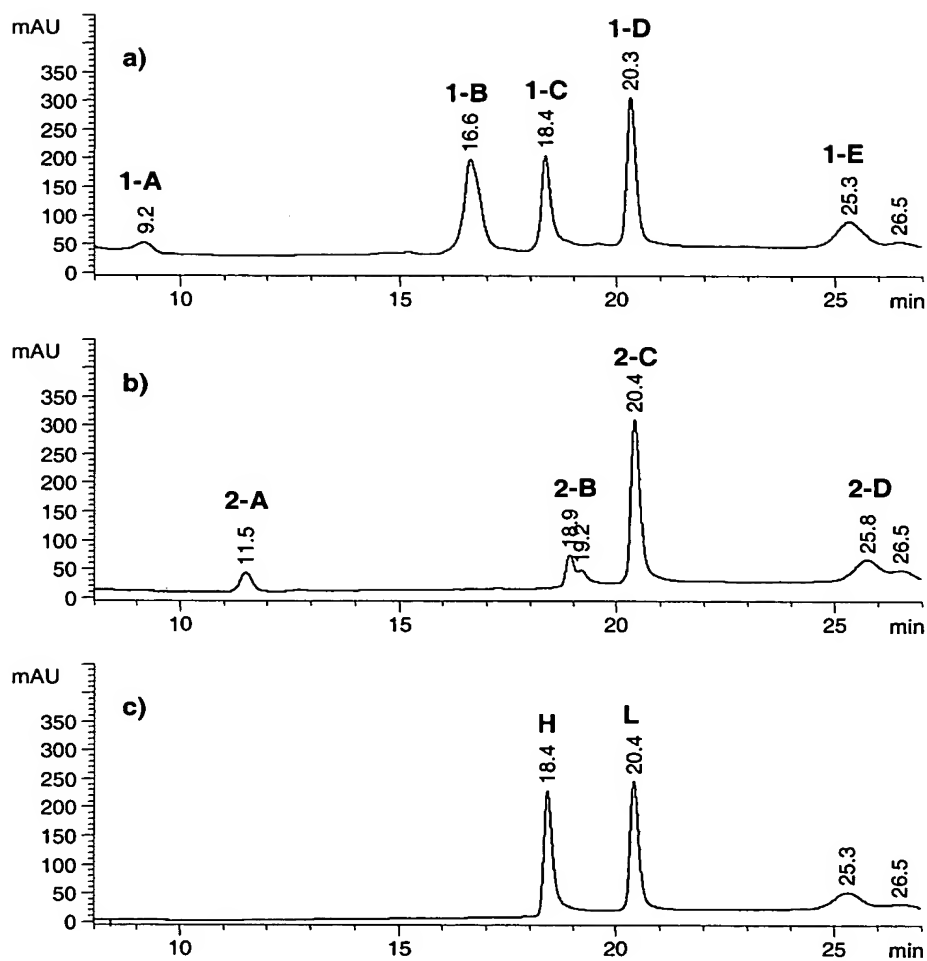
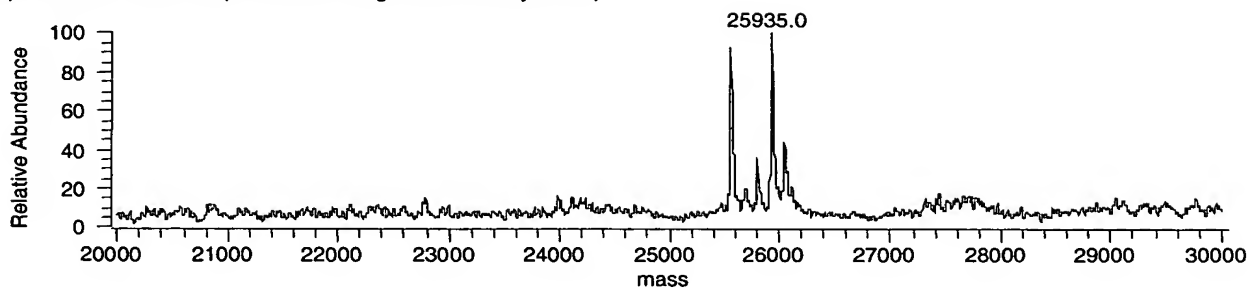
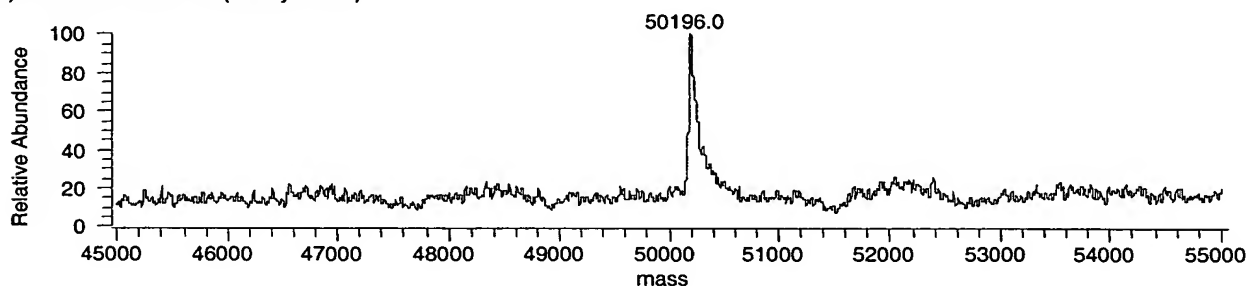


Figure 6: RP-HPLC chromatography for LC-ESI-MS analysis of deglycosylated, reduced, and alkylated: a) SEC fraction 'Peak 1', b) SEC fraction 'Peak 2', and c) non-heated reference standard of MAb A.

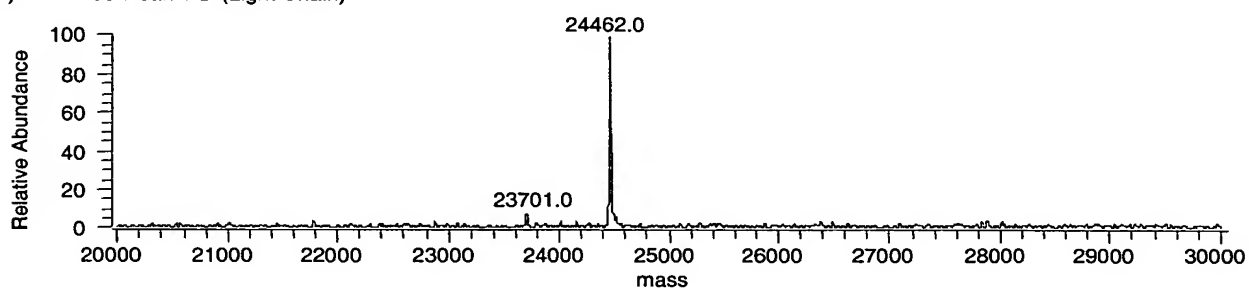
a ) RP-HPLC Peak 1-B (C-Terminal Fragment of Heavy Chain)



b ) RP-HPLC Peak 1-C (Heavy Chain)



c ) RP-HPLC Peak 1-D (Light Chain)



d ) RP-HPLC Peak 1-E (Heavy Chain)

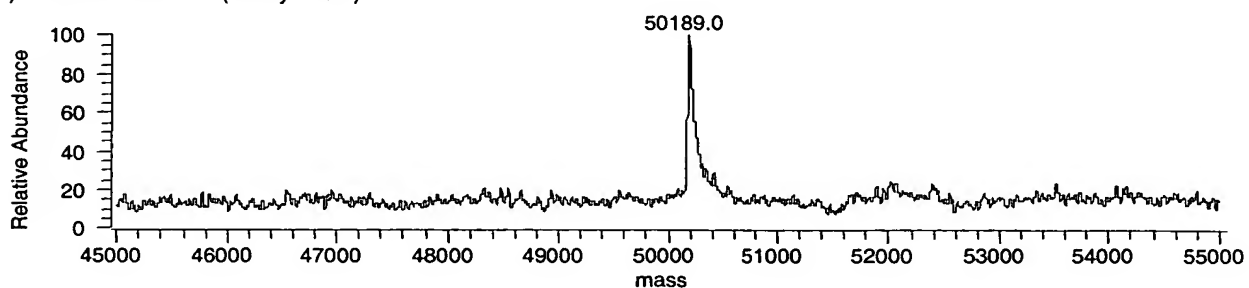


Figure 7: Reconstructed ESI-MS mass spectra for each peak a) 1-B, b) 1-C, c) 1-D, and d) 1-E shown in RP-HPLC chromatogram of deglycosylated, reduced, and alkylated SEC fraction 'Peak 1' of MAb A (Figure 6a).

a ) RP-HPLC Peak 1-B (C-Terminal Fragments of Heavy Chain)

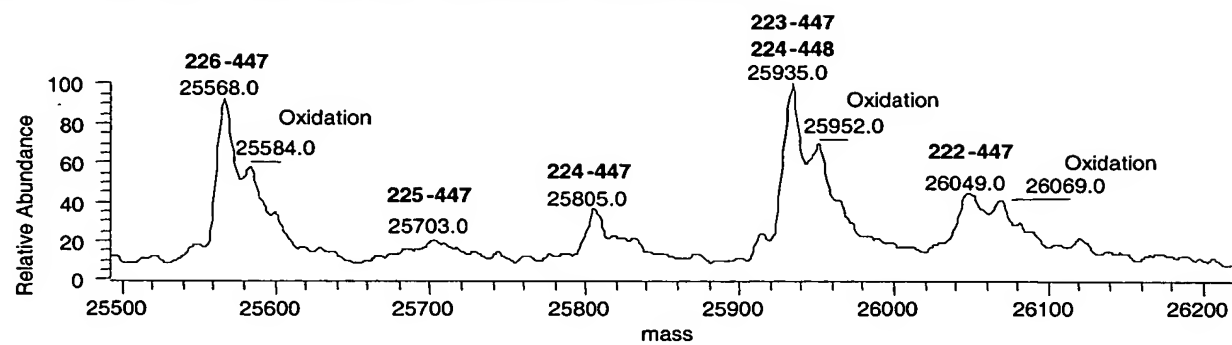
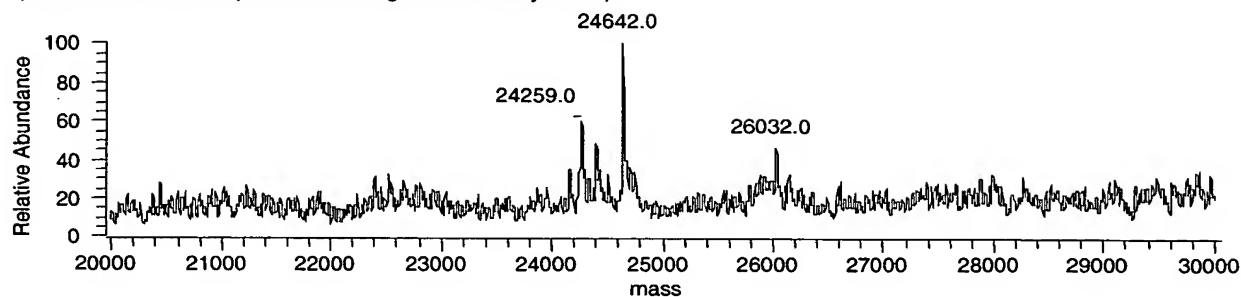
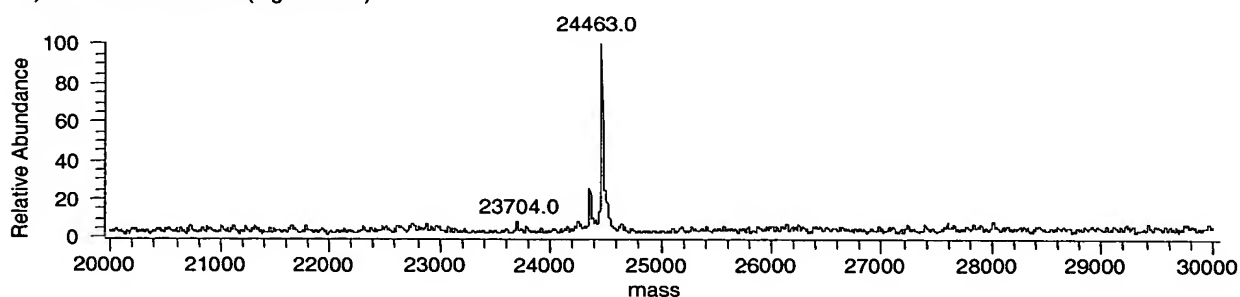


Figure 8: Expansion of the reconstructed mass spectrum shown in Figure 7a.

a ) RP-HPLC Peak 2-B (N-Terminal Fragment of Heavy Chain)



b ) RP-HPLC Peak 2-C (Light Chain)



c ) RP-HPLC Peak 2-D (Light Chain & N-Terminal Fragment of Heavy Chain)

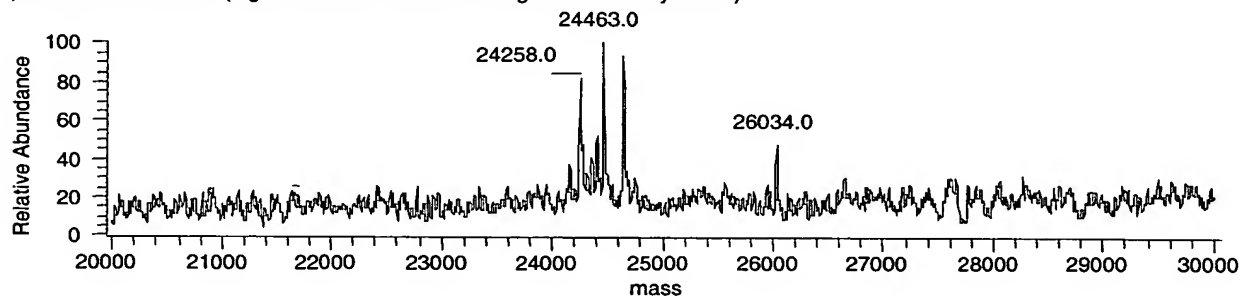


Figure 9: Reconstructed ESI-MS mass spectra for each peak a) 2-B, b) 2-C, and c) 2-D, shown in RP-HPLC chromatogram of deglycosylated, reduced, and alkylated SEC fraction 'Peak 2' of MAb A (Figure 6b).

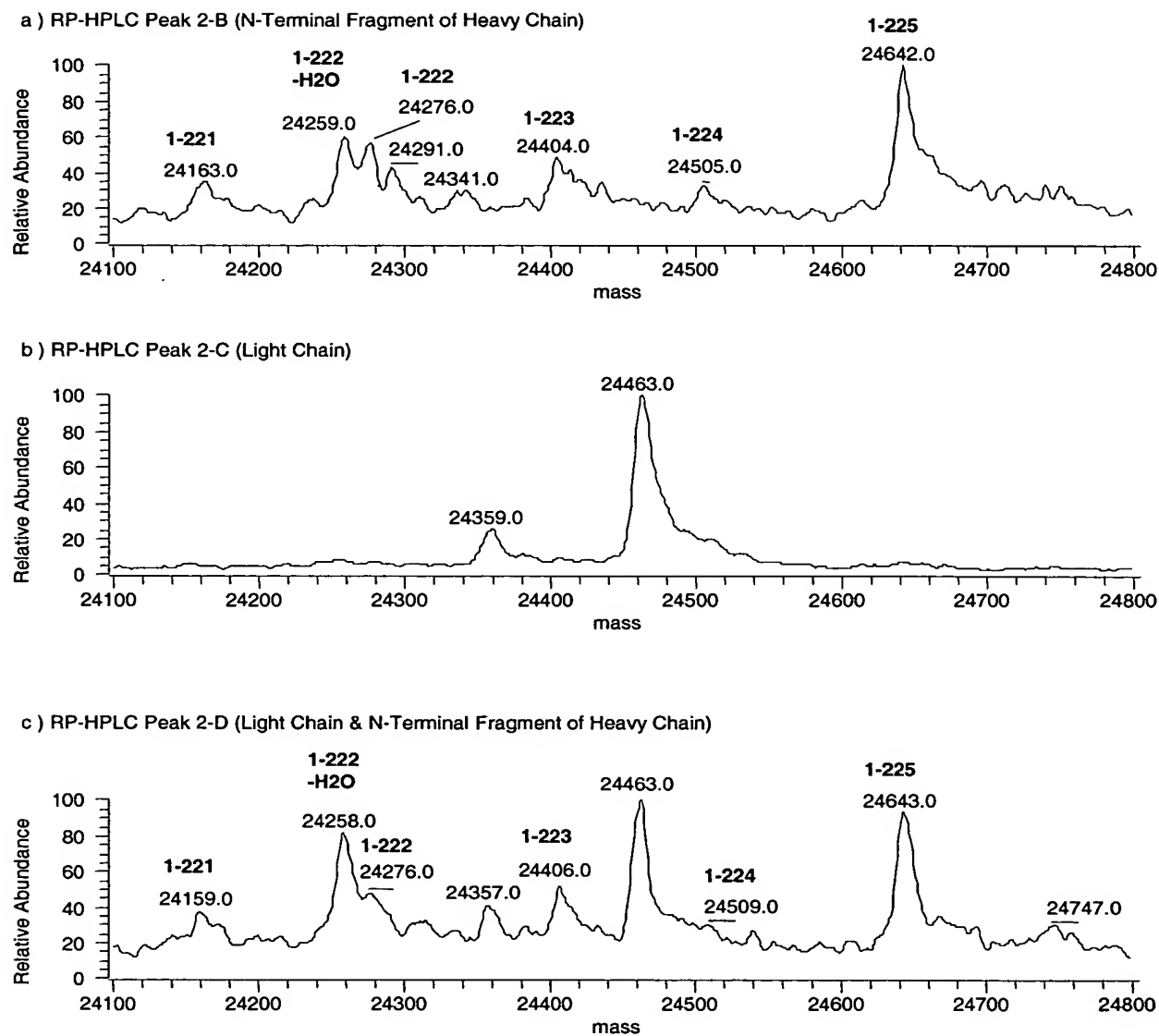


Figure 10: Expansion of the reconstructed mass spectra shown in Figure 9.



# Experimental Diagram

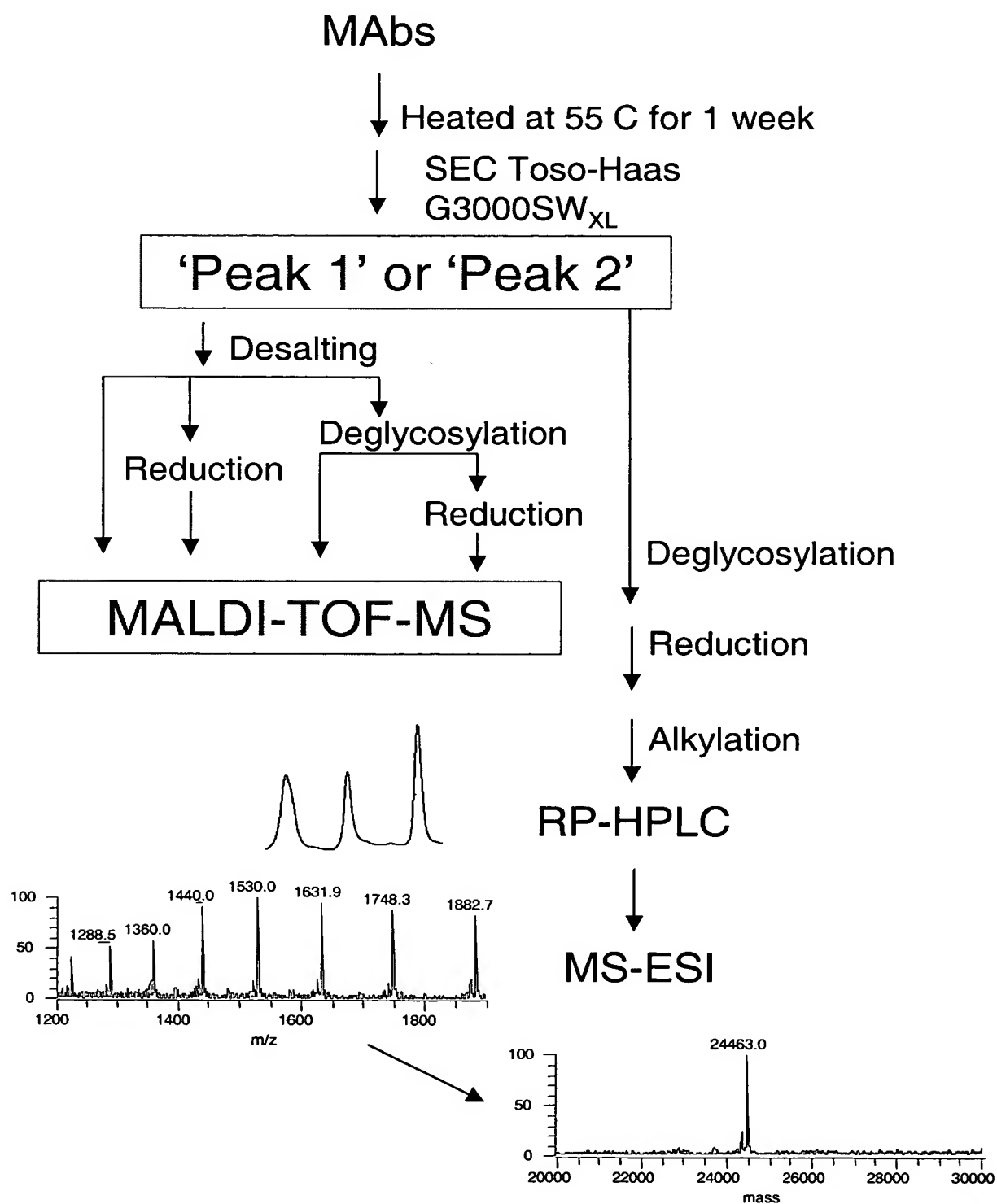


Figure 11

## Heat-induced fragmentation of MAbs

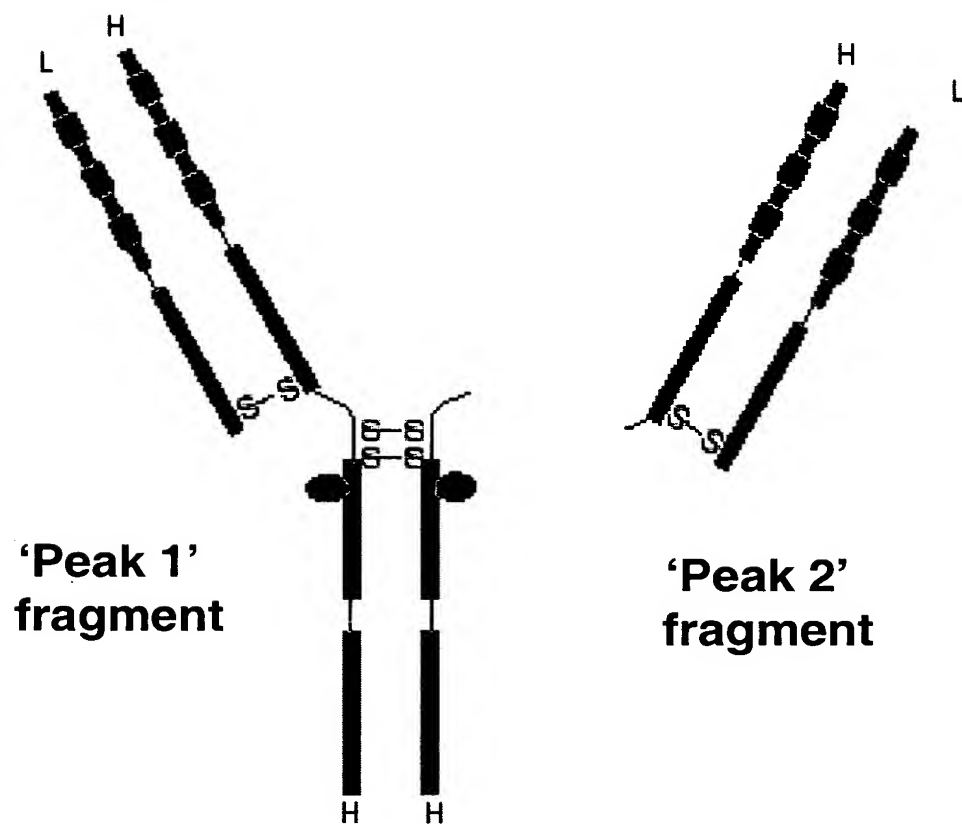


Figure 12